Università degli Studi di Siena

Dipartimento di Biotecnologie, Chimica e Farmacia

DOTTORATO DI RICERCA IN SCIENZE CHIMICHE E FARMACEUTICHE

CICLO **XXIX**

COORDINATOR: **Prof. Stefano Mangani**

TITLE OF THE THESIS:

Synthesis and characterization of new polymeric materials for pharmaceutical and optoelectronic applications

TUTOR: **Prof. Andrea Cappelli**

CANDIDATE: **Dr. Vincenzo Razzano**

ACADEMIC YEAR: **2016/2017**

Synthesis and characterization of new polymeric materials for pharmaceutical and optoelectronic applications

Chapter 1.

Introduction

Polymers

Definition and properties

Polymers are high molecular weight macromolecules characterized by a sequence of monomeric repeating units linked each other by covalent bonds. The number of structural units contained in a macromolecule is the degree of polymerization (DP). The molecular weight of a polymer is given by the product of DP with the molecular weight of the repeating unit.[1]

Polymers made by the repetition of a single structural unit are called homopolymers (-A-A-A-A-A-A-A-A-), whereas the combination of different monomers leads to copolymers (-A-B-A-A-A-B-B-A-). The latter macromolecules can be classified in alternating (-A-B-A-B-A-B-A-B-), random (-A-B-A-A-A-B-B-A-) and block (-A-A-A-A-B-B-B-B-) copolymers.

Furthermore, polymer chains can even assume both linear, branched or crosslinked architectures (Figure 1).[2]

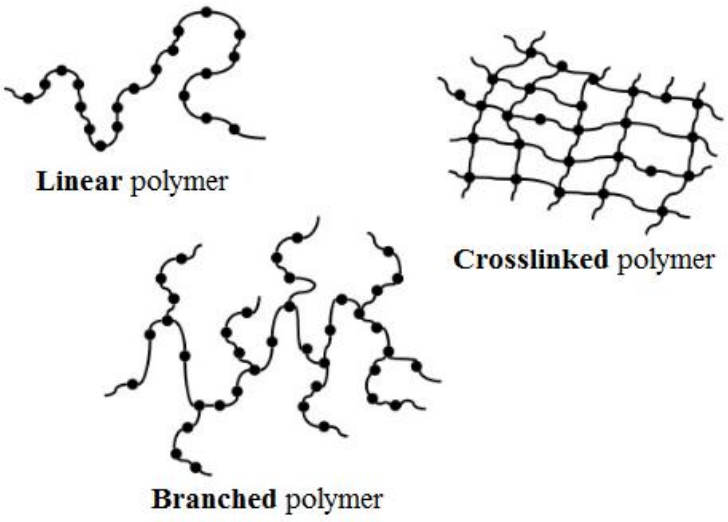

Figure 1. Schematic illustration of polymer chains.

The presence of asymmetric elements in the polymer structure (i.e. chiral carbons, double bonds) and the way they are oriented in the space are responsible for the different polymer configurations. Some of them can show a certain degree of repetitiveness (tacticity) along the polymer chain (i.e. *cis* configuration for all the double bonds, *R* configuration for all the asymmetric carbons). On the basis of these considerations, polymers are defined isotactic, syndiotactic and atactic (Figure 2).

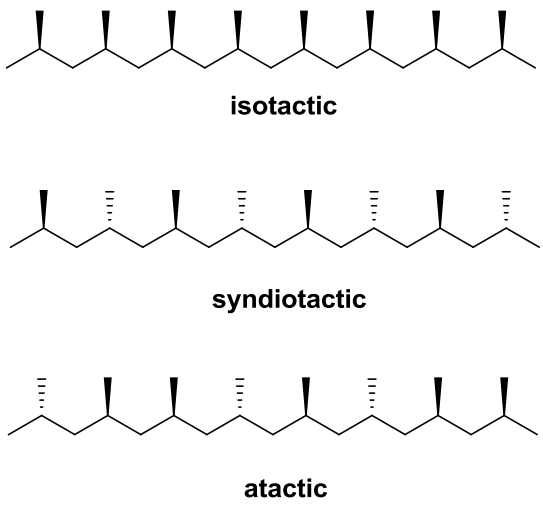

Figure 2. Examples of atactic, isotactic and syndiotactic polymers.

Tacticity significantly affects the chemo-physical and mechanical properties of chiral polymers (i.e. solubility, viscosity, elasticity, flexibility, stiffness, melting point, crystallinity, etc.).
Only few polymers have a completely crystalline architecture, but there are totally amorphous. Generally, they are semi-crystalline solids in which crystalline regions and amorphous domains can be distinguished (Figure 3).[3]

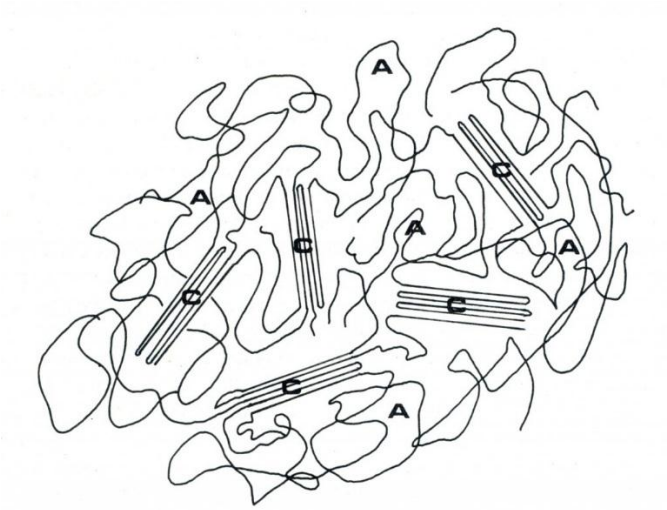

Figure 3. Amorphous (**A**) and crystalline (**C**) regions in a polymer. The crystalline regions showed ordered arrangements of polymer chains.

The polymer amorphous state can be characterized by the typical features of glassy or rubbery materials as a function of their glass transition temperature (Tg). Above Tg the polymer is in the rubbery state, showing elastic properties (elastomers). On the other hand, below Tg the polymer is in the glassy state (thermoplastics). Elastomers are elastic and flexible; in fact the kinetic energy of the systems is sufficient to allow the various macromolecule zones to move. Differently, thermoplastic

polymers are hard and brittle and polymer chains appear “frozen” in their positions and not prone to deformation.[2]

Many of the above mentioned polymer properties are closely related to the molecular weight. In particular, due to the nature of the growth processes all synthetic polymers appear as polydisperse materials consisting of polymer chains of different length. For this reason, the molecular weight doesn’t assume a fixed value, but it exists as a distribution of molecular weights characterized by an average value and a variance (Figure 4).[4]

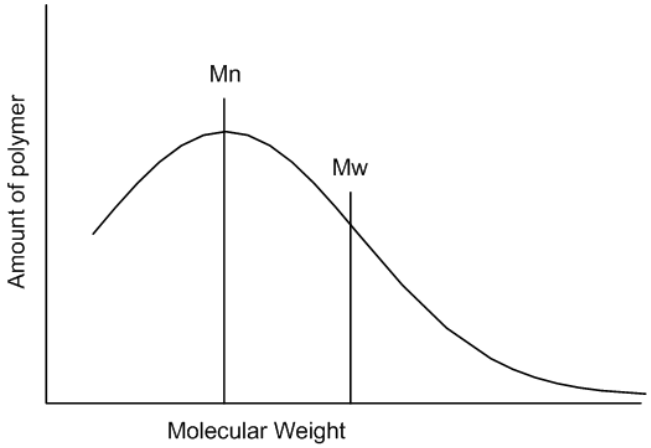

Figure 4. Schematic plot of the typical molecular weight distribution (MWD) of a polymer.

The molecular weight of a polymer can be described as an average of the molecular weights of all the chains in the sample. The most commonly used molecular weight averages for a polymer are:

1) The number average molecular weight (Mn) represents the arithmetic average molecular weight (Mi) of all the polymer chains (Ni);[4]

$$Mn = \frac{\Sigma N_i M_i}{\Sigma N_i}$$

2) The weight average molecular weight (Mw) takes into account the molecular weight of a chain in determining contributions to the molecular weight average. This value tends to highlight especially with the participation of heavier macromolecules. The more massive the chain, the more the chain contributes to Mw.[4]

$$Mw = \frac{\Sigma N_i M_i^2}{\Sigma N_i M_i}$$

The Mw/Mn ratio corresponds to the polydispersity index (PDI) and can be used as a measure of the broadness of the molecular weight distribution (MWD) of a polymer. PDI can be equal or greater than 1 and the larger its value, the broader the dispersion. A monodisperse polymer where all the chain

lengths are equal (such as a protein) has an Mw/Mn = 1. On the other hand, a polydisperse polymer where the macromolecules are less uniformly dispersed (such as a narrow synthetic polymer used for calibrations) has an Mw/Mn of 1.02 to 1.10.[4]

Types of polymers and polymerizations

During the development of polymer science, two types of classifications have come into use. One classification is based on polymer structure and divides polymers into condensation and addition polymers. The other classification is based on polymerization mechanism and divides polymerizations into step and chain polymerizations. Generally, most condensation polymers are produced by step polymerizations and most addition polymers are produced by chain polymerizations.[5]

Condensation polymers.

Condensation polymers are composed of polyfunctional monomers, which are involved in the various condensation reactions with the elimination of small molecules such as water or HCl. An example of such condensation polymers are polyesters, formed by dialcohols and diacids with the elimination of water (Figure 5).[5]

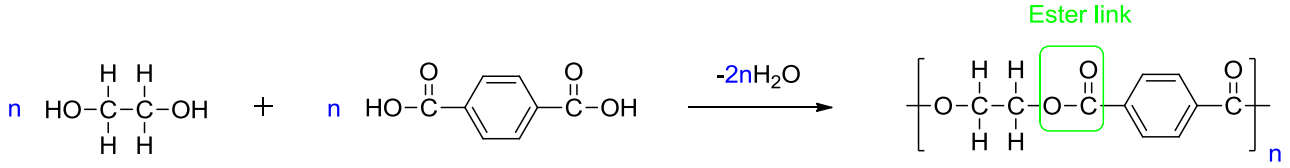

Figure 5. Example of one type of condensation polymer.

Addition polymers.

Addition polymers are formed by a reaction known as polyaddition or addition polymerization, where many unsaturated monomers bond together via rearrangement of bonds without losing any atoms or molecules (Figure 6). This process can occur in a variety of ways including:

1) Free radical polymerization, which is a method of polymerization by which one or two radicals are created by the attack of suitably activated (Δ, hν, redox, current, ultrasounds) radical initiators (i.e. various peroxides, AIBN, etc.) that start the polymerization process. These reactive species add to a monomer molecule by opening the π-bond to form a new radical. Once a chain has been initiated, the chain propagates until termination occurs.[5]

2) Ionic polymerization, which is a type of chain-growth polymerization where reactive centers are ions instead of free radicals. Depending on the charge type (positive or negative), this polymerization can be defined as cationic or anionic polymerization. Lewis acids and nucleophilic compounds (organometals, $NaNH_2$, alkoxides, amines, phosphines, etc.) are commonly used as initiator systems in ionic polymerization.[5]

3) Coordination polymerization, which is a form of addition polymerization where monomers add to a growing macromolecule by means of the coordination complex formation with an organometallic active center (such as the Ziegler-Natta catalyst). Polymers obtained with this technique tend to be stereoregular and can be atactic, isotactic or syndiotactic, showing similar features to the anionic polymerization.[5]

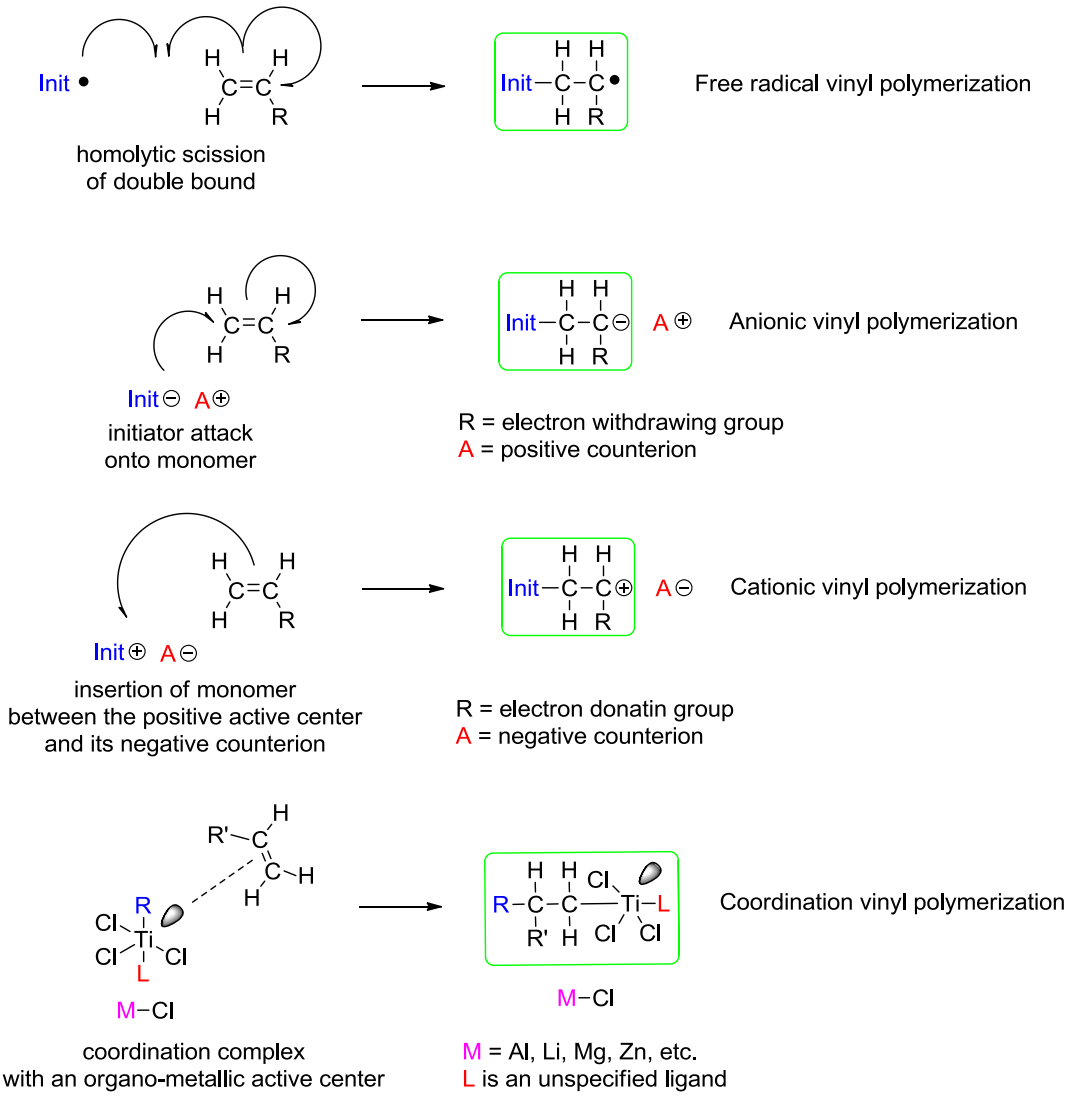

Figure 6. Comparison of radical, anionic, cationic and coordination polymerizations.

Step and chain polymerizations.

These synthetic techniques can be defined more accurately by examining the mechanism by which they proceed. In fact, this assembly process can occur with two growth mechanisms: step and chain polymerization. In step polymerization, monomers are quickly depleted by transforming in longer reactive species (dimers, trimers, tetramers and so on). The size of molecules increases at a relatively slow pace in such polymerizations. On the other hand, long and high molecular weight chains form rapidly in chain polymerization where the monomer concentration is high throughout the reaction because only species with reactive centers add monomers one by one (Figure 7).[5]

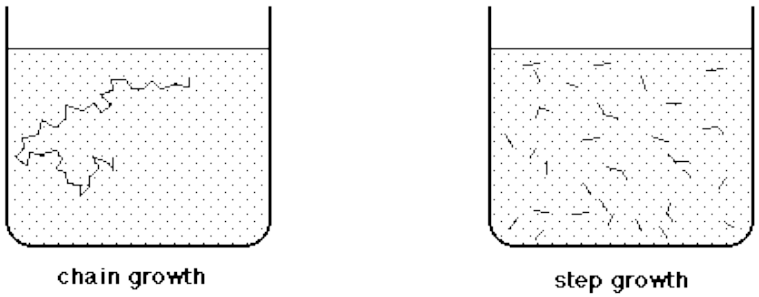

Figure 7. Molecular size distributions for step and chain growth polymerizations.

Many anionic polymerizations take place under conditions where there are no effective termination reactions. This allows to obtain polymeric materials showing narrow dispersions. On the other hand, relatively broad molecular weight distributions are generally encountered in cationic conditions. In fact, the latters are more sensitive to the presence of impurities due to the high reactivity of carbocation centers determining also a faster kinetic compared to both anionic and free radical processes.[5]

In particular, addition polymers are characterized by three different termination events. Free radical polymers can usually quench by combining each other after coming in close proximity or by disproportionation reactions (hydrogen atom from one chain end is abstracted to another, producing a polymer with a terminal unsaturated group and a polymer with a terminal saturated group). On the contrary, the ionic propagations are never terminated by bimolecular couplings between two growing chains because of their electric repulsion.[5]

Termination in cationic polymerizations generally occurs via unimolecular rearrangement with the counterion. In this process an anionic fragment of the counterion combines with the propagating carbocation. This not only inactivates the growing chain, but it also terminates the kinetic chain by reducing the concentration of the initiator/co-initiator complex. Another important chain-breaking reaction in cationic polymerizations is the β-proton transfer (or chain transfer), which can take place

in two ways. One method of chain transfer involves hydrogen abstraction from the active chain end to the counterion. In this case the growing chain is terminated, but the H^+-counterion complex is generated to initiate more chains. The second method involves hydrogen abstraction from the active chain end to the monomer. This terminates the growing chain and also forms a new active carbocation-counterion complex which can continue to propagate. Other transfer, combination or termination events involving solvents, impurities or agents deliberately added to the reaction system can also occur. Anyway, these quenching pathways are less favourable for the anionic polymerization. Their polymerization conditions don't show any intrinsic termination mechanisms unless external quenching agents are added to the system (i.e. alcohols, acids and epoxides which then become end groups).[5] Interestingly, such polymer chains are demonstrated to keep growing until the complete depletion of monomers because of the absence of any intrinsic mechanisms of termination.[6] Provided that inadvertent or deliberate chain-breaking reactions don't occur, suitably stored carbanions may survive indefinitely (immortal polymers). As a consequence, the propagation can be restarted at any time by adding more monomers to the system.

This surprising behaviour is typical of the so called living polymerization (LP), which is a popular method for synthesizing block copolymers since a polymer can be synthesized in stages, each stage containing a different monomer. This method is more desirable for materials design because it offers precision and control in macromolecular synthesis. Additional advantages are predetermined molar mass and control over end-groups, since molecular weight and dispersity are less controlled in non-living polymerizations.[5,7] However, living polymers don't live forever because the concentration of carbanion centers decays with time also in the absence of terminating agents.[5] It is very difficult to realize living cationic polymerizations because of the high reactivity shown by carbocations, even in the best purified system lacking of any nucleophiles.[5]

In the last years, new methods were developed which enabled an adaptation of living ionic polymerization to living radical polymerization (LRP), also referred to as controlled radical polymerization (CRP). CRP has branched into three fundamental techniques including atom-transfer radical polymerization (ATRP), stable free-radical polymerization (SFRP) and reversible addition–fragmentation transfer (RAFT). In particular, ATRP and SFRP proceed with reversible termination, while RAFT proceeds with reversible chain transfer. These techniques are based on the persistent radical effect (PRE) suppressing bimolecular termination and have a good commercial potential for materials synthesis because many more monomers can undergo radical polymerization compared to ionic routes.[5]

Applications of polymers in biomedical field

Polymers are a promising class of materials that can be engineered to meet specific end-use requirements. Applications include surgical devices, implants and supporting materials (i.e. artificial organs, prostheses and sutures), drug-delivery systems with different routes of administration and design, carriers of immobilized enzymes and cells, biosensors, components of diagnostic assays, bioadhesives, ocular devices, and materials for orthopedic applications. The selection and design of a polymer is a challenging task because of the inherent diversity of structures and thus it requires a thorough understanding of the surface and bulk properties of the polymer that can give the desired chemical, physical, interfacial, mechanical and biological functions. In addition to its physical-chemical properties, the choice of a polymer is dependent on the need for extensive biochemical characterization and specific preclinical tests to prove its safety.[8]

Polymers used as biomaterials can be natural, synthetic or a combination of both. Table 1 gives a representative list of the main groups of polymeric materials. [9]

Natural polymers are usually biodegradable and offer excellent biocompatibility, but they suffer from batch to batch variations because of the lack of reproducible production methods and difficulties in purification. On the other hand, synthetic polymers are available in a wide variety of compositions with readily adjustable properties. Although the primary difficulty of the majority of synthetic materials is the general lack of biocompatibility, in which poly(ethylene oxide) (PEO) and poly(lactic-co-glycolic acid) are notable exceptions, the use of synthetic polymers overcomes above disadvantages and hence their use is preferred.[8]

There are various synthetic biodegradable polymers currently being investigated as drug delivery systems or as scaffolds for tissue engineering. Biodegradable polymers are mainly used where the transient existence of materials is required and they find applications as sutures, scaffolds for tissue regeneration, tissue adhesives, hemostats, transient barriers for tissue adhesion, as well as drug delivery systems.[10] On the contrary, materials for long term use (orthopedic and dental implants) must be water-repellent to avoid degradation or erosion processes that lead to changes in toughness and loss of mechanical strength.[8] Each of these applications demands materials with unique physical, chemical, biological, and biomechanical properties to provide efficient therapy.

Table 1. Representative list of the main groups of polymeric materials.[9]

Classification	Polymer
Natural polymers	
Protein-based polymers	Collagen, albumin, gelatin
Polysaccharides	Agarose, alginate, carrageenan, hyaluronic acid, dextran, chitosan, cyclodextrins
Synthetic polymers	
Biodegradable	
Polyesters	Poly(lactic acid), poly(glycolic acid), poly(hydroxy butyrate), poly(ε-caprolactone), poly(β-malic acid), poly(dioxanones)
Polyanhydrides	Poly(sebacic acid), poly(adipic acid), poly(terphthalic acid) and various copolymers
Polyamides	Poly(imino carbonates), polyamino acids
Phosphorous-based polymers	Polyphosphates, polyphosphonates, polyphosphazenes
Others	Poly(cyano acrylates), polyurethanes, polyortho esters, polydihydropyrans, polyacetals
Non-biodegradable	
Cellulose derivatives	Carboxymethyl cellulose, ethyl cellulose, cellulose acetate, cellulose acetate propionate, hydroxypropyl methyl cellulose
Silicones	Polydimethylsiloxane, colloidal silica
Acrylic polymers	Polymethacrylates, poly(methyl methacrylate), poly hydro(ethyl-methacrylate)
Others	Polyvinyl pyrrolidone, ethyl vinyl acetate, poloxamers, poloxamines

Drug Delivery Systems

A major challenge in drug development is making sure that each new candidate is delivered to the right place, at the right time and in the right amount. Low drug solubility, drug degradation, drug toxicity, or rapid clearance from the body can reduce the effectiveness of an otherwise promising candidate.

In the last years, polymers have played an integral role in the advancement of drug delivery technology by providing controlled release of therapeutic agents in constant doses over long periods, cyclic dosage, and tunable release of both hydrophilic and hydrophobic drugs. Although conventional drug delivery formulations have greatly contributed to the treatment of diseases, the emergence of potent and specific biological therapeutics has escalated the impetus for intelligent delivery systems.

These intelligent delivery systems must address the need for specific targeting, intracellular transport, and biocompatibility while integrating elements of responsive behavior to physiological environments and recognitive feedback control.[11]

The central objective of a delivery system is to release therapeutics at the desired anatomical site and to maintain the drug concentration within a therapeutic band for a desired duration (Figure 8).[11]

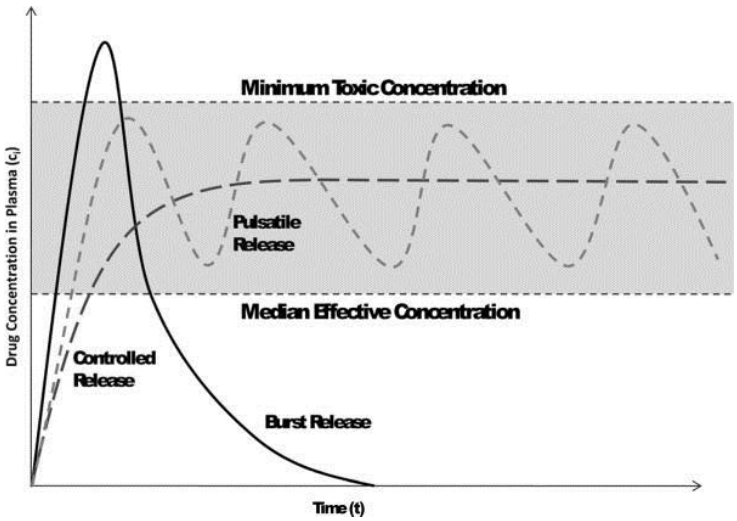

Figure 8. Therapeutic band with showing impact of burst release, pulsatile release, and controlled release relative to effective concentration and toxic concentration.[11]

For more than 50 years, techniques such as compression, spray and dip coating, and encapsulation have been used in the pharmaceutical industry to incorporate bioactive agents within polymers.[11]

In order to be used for controlled drug delivery formulations, the polymers must be chemically inert and free of leachable impurities with appropriate physical structure, minimal undesired aging, and be readily processable. Some examples are polylactic acid (PLA), polyglycolic acid (PGA), poly(lactic-co-glycolic acid) (PLGA), poly-1-glutamic acid, poly(N-isopropylacrylamide), poly(2-hydroxyethyl methacrylate), poly(N-vinyl pyrrolidone), polypyrrole, poly(amidoamine) (PAMAM), poly(ethylene glycol) (PEG), dextran.[12,13]

From a drug delivery perspective, polymer devices can be categorized as diffusion-controlled (monolithic devices), solvent-activated (swelling devices), biodegradable and bioerodible, or externally-triggered systems (e.g., pH, temperature).[11]

Diffusion-controlled.

Most diffusion-controlled carriers are simple and monolithic in nature. In these systems, a drug is dissolved in a non-swellable system or fully swollen matrix which does not decompose during its therapeutic life.[11]

Solvent-activated.

In solvent-activated systems like hydrogels, drugs are loaded into dehydrated polymers by simply packing the two substances together. They are hydrophilic in nature. Once exposed to an aqueous environment, the hydrogels imbibe water, swell and release the drug.[11]

Biodegradable and bioerodible.

Biodegradable and bioerodible polymers represent an important class of materials for drug delivery. Although often used interchangeably, degradation and erosion are different processes. In bioerodible polymers, erosion occurs by the dissolution of chain fragments in non-crosslinked systems without bringing any change to the molecular structure. Differently, biodegradable polymers break due to cleavage of covalent bonds between them by means of chemical reactions that occurs in degradation. To obtain dissolution, the polymer must absorb the surrounding aqueous solvent and must interact with water via charge interactions (such as with polyacids and polybases) or hydrogen bonding mechanisms. To be chemically degradable, polymers require hydrolytically or proteolytically labile bonds in their backbone or crosslinker. The majority of biodegradable synthetic polymers include ester derivatives such as poly(lactic-co-glycolic acid).[11]

Externally-triggered.

Externally-triggered polymers, or smart polymers, are a class of materials comprising a large variety of linear and branched copolymers or crosslinked polymer networks. A hallmark of responsive polymers is their ability to undergo a dramatic physical or chemical change in response to external stimuli. Temperature and pH changes are commonly used to trigger behavioral changes, but other stimuli, such as ultrasound, ionic strength, redox potential, electromagnetic radiation, and chemical or biochemical agents, can be used. These stimuli can induce a response by altering molecular interactions between polymer and solvent (adjusting hydrophobic/hydrophilic balance) or between polymer chains (influencing crosslink or backbone integrity, proclivity for hydrophobic association, or electrostatic repulsion).[11] Types of behavioral change can include dissolution, precipitation, swelling, change in conformation, and change in hydrophobic and hydrophilic properties. The polymer composition, the nature of the ionizable groups, the hydrophilicity of the polymer backbone and the crosslinking density decide the behaviour of the smart polymers. Alteration in polymer properties can be used to adhere to the cell surface, break down cellular membrane, or release biologically active compounds.[13]

In order to minimize drug degradation and loss, to prevent harmful side-effects and to increase drug bioavailability and the fraction of the drug accumulated in the required zone, various polymer carriers

including micro-/nano-spheres, micro-/nano-capsules, hydrogels, micelles, liposomes and polymer-drug conjugates have been designed (Figure 9).[14]

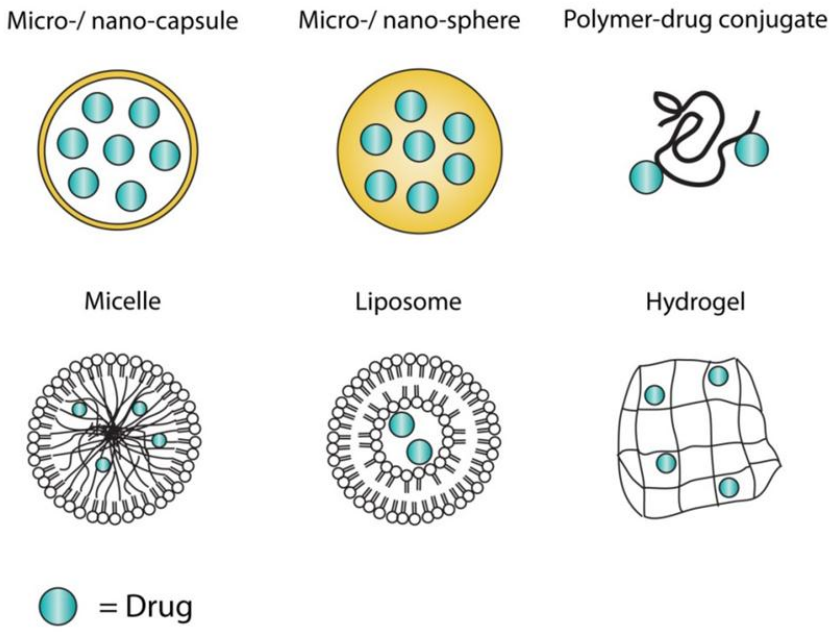

Figure 9. Different structures and morphology of DDSs.[14]

Furthermore, polymers serving as drug carrier should be water soluble, non-toxic and non-immunogenic. They work passively in minimizing drug degradation and improving circulation time. If the polymer is non-degradable, it should be ensured that it is not accumulated within the body and, if it is degradable, the broken components should be non-toxic and such that they lie below the renal threshold level and should not produce any immune response.[13]

Micro-/nano-spheres and micro-/nano-capsules.

Depending on their composition polymeric micro-/nano-particles can be distinguished in micro-/nano-spheres and micro-/nano-capsules. In particular, micro-/nano-spheres are matrix systems in which the drug is dispersed within the polymer throughout the body of the particle. Differently, micro-/nano-capsules are vesicular systems in which the cavity containing the drug (oily/aqueous core) is surrounded by a single ultrathin polymer membrane (reservoir systems for controlled drug release). Drug release from polymeric micro-/nano-particles occurs by diffusion through the polymer or by degradation of the polymer.[13]

Hydrogels.

Hydrogels are hydrophilic crosslinked networks capable of imbibing large amounts of water or

biological fluids. Physical or covalent crosslinks render hydrogels with high porosity. Tailored porous hydrogels can be obtained by controlling the level of cross-linking, which in turn affect the release rate of the entrapped drug molecules. Hydrogels can be made from both natural and synthetic polymers. Biodegradable hydrogels are used as carriers for controlled drug delivery because of their inertness for many drugs and their biocompatibility.[13]

Micelles.

Polymeric micelles have a core shell structure formed by spontaneous self-assembly of individual amphiphilic di/tri block copolymers. They have both hydrophilic and hydrophobic regions. The core of a micelle is formed by hydrophobic block polymer blocks while the shell is made up of hydroplilic polymer.[13] The drugs can be physically entrapped in the core of block copolymer micelles and transported at concentrations that can exceed their intrinsic water-solubility. Moreover, the hydrophilic blocks can form hydrogen bonds with the surrounding aqueous environment and form a tight shell around the micellar core. As a result, the contents of the hydrophobic core are effectively protected against hydrolysis and enzymatic degradation. In addition, the corona may prevent recognition by the reticulo-endothelial system and therefore, preliminary elimination of the micelles from the bloodstream.[12]

Liposomes.

Liposomes are artificial vesicles composed of one or more concentric phospholipids bilayers. They are amphiphilic. Liposomes can trap both hydrophobic and hydrophilic compounds. The internal aqueous core is suited to accommodate hydrophilic drugs while the phospholipid bilayer entraps hydrophobic drugs. The modification of the liposomal surface by attaching dextran or PEG to the phospholipid bilayer increases their circulation time in the bloodstream.[13]

Because of their biocompatibility, biodegradability, low toxicity, and aptitude to trap both hydrophilic and lipophilic drugs and simplify site-specific drug delivery to tumor tissues, numerous different liposome formulations of numerous anticancer agents were shown to be less toxic than the free drug.[15]

Polymer-drug conjugates.

Polymer-drug conjugates consist of a biocompatible polymer backbones bound to three components: a solubilizer, which serves to impart hydrophilicity and ensure water-solubility; a drug, usually bound to the polymeric backbone via a linker; and a targeting moiety, whose function is to provide transport to a desired physiological destination or bind to a particular biological target.

These polymer-drug conjugates offer several significant advantages over traditional small molecule

therapeutics. First, the aqueous solubility of a drug can be dramatically improved following conjugation to a water-soluble polymer. Next, polymer-drug conjugates offer the potential for a drug to be delivered in a controlled manner over a defined time interval (there is a physiological labile bond between the drug and the polymer). As previously mentioned, another major advantage that can be realized through drug-polymer conjugation is the inclusion of targeting moieties, which function to carry the drug to the site of pharmacological action.[16]

Dendrimers

Although conventional drug delivery systems are useful in overcoming limitations of several therapeutic agents, like low aqueous solubility and short half-life, they have failed to prove their effectiveness in delivering many drugs. Moreover, the traditional linear polymers (i.e. polyethylene glycol (PEG), polyglutamic acid, polysaccharide, etc) have been reviewed as drug delivery vehicles because of their poorly defined chemical structures. In this scenario, many attempts have been made to alleviate these problems associated with them. In addition, a considerable interest of scientists in delivering therapeutic, targeting and diagnostic agents together in a single system has led to the usage of a novel class of nanoparticles as multifunctional platforms.

Dendrimers are emerging polymeric architectures that are known for their defined structures, versatility in drug delivery and high functionality whose properties resemble those of biomolecules.[17] The first pioneering synthetic attempt in building dendritic structures was made by Fritz Vögtle and coworkers (1978)[18] by using cascade reactions both in a convergent and divergent approach.[19]

Various dendritic platforms such as polyamidoamine (PAMAM), poly(propylene imine) (PPI), poly-L-lysine, melamine, poly(etherhydroxylamine) (PEHAM), poly(esteramine) (PEA) and polyglycerol have been synthesized and explored as drug delivery vehicles.[17]

Dendrimers are three-dimensional, hyperbranched, monodisperse macromolecules showing nano-size dimensions (Figure 10). They consist of three structural components: central core (multifunctional), branched units and surface groups.[13]

They are broadly used as potential site-specific delivery systems for drugs and other therapeutic agents. In particular, they can act as carriers (vectors) in anticancer, antiviral, antibacterial and gene therapy.[20] The peripheral residues can be functionalized as needed by linking therapeutic agents,[20] targeting (i.e. folic acid)[21,22] or solubilizing moieties (i.e. PEG and sugars)[20] and imaging/diagnostic probes (i.e. gadolinium)[20] in a predictable, reproducible and controlled manner. Furthermore, small bioactive molecules can be also physically entrapped in the interior of the dendrimers by the establishment of host-guest interactions.[13]

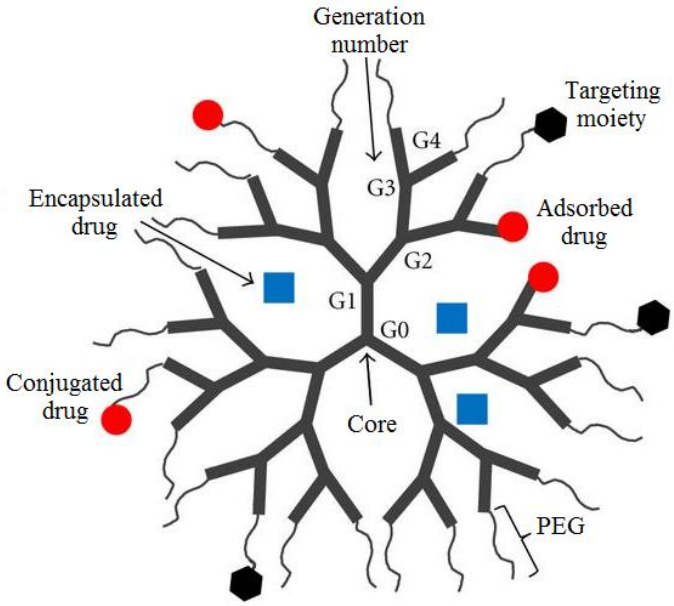

Figure 10. Simultaneous delivery of hydrophilic and hydrophobic drugs by encapsulation within hydrophobic cavities inside branching clefts, adsorption to the surface (ionic interaction) or direct covalent conjugation with functional groups on the surface.[13]

Polymer brushes

A polymer brush consists of end-tethered (or end-grafted) polymer chains stretched away from the substrate so that the brush height in the given solvent is greater if compared to the end-to-end distance of the same non-grafted chains dissolved in the same solvent. The substrates can be in any physical state: solid, liquid, or gas. Tethering of polymer chains on the substrate can be realized through covalent bonds, electrostatic interactions, hydrogen bonds, or strong van der Waals interactions. However, weak interactions are less interesting.[23]

Among the many biomolecules at work in nature there is a group known as proteoglycans which show a peculiar brush-like architecture. Proteoglycans are macromolecules that consist of a peptide backbone bearing polysaccaride side chains. These molecules are found in different places in the human body performing both structural (extracellular matrix) and cell signaling functions. In particular, they act as water sponges in cartilage controlling its resilience and lubrication properties. Furthermore, they affect the mucociliary clearance of lung airways by controlling mucus viscoelasticity. These functional properties of proteoglycans are generally believed to be related to their brush-like architecture that ensures dense packing of functional groups along the backbone.[24] One important proteoglycan is aggrecan (Figure 11) which is the major constituent of the articular cartilage.

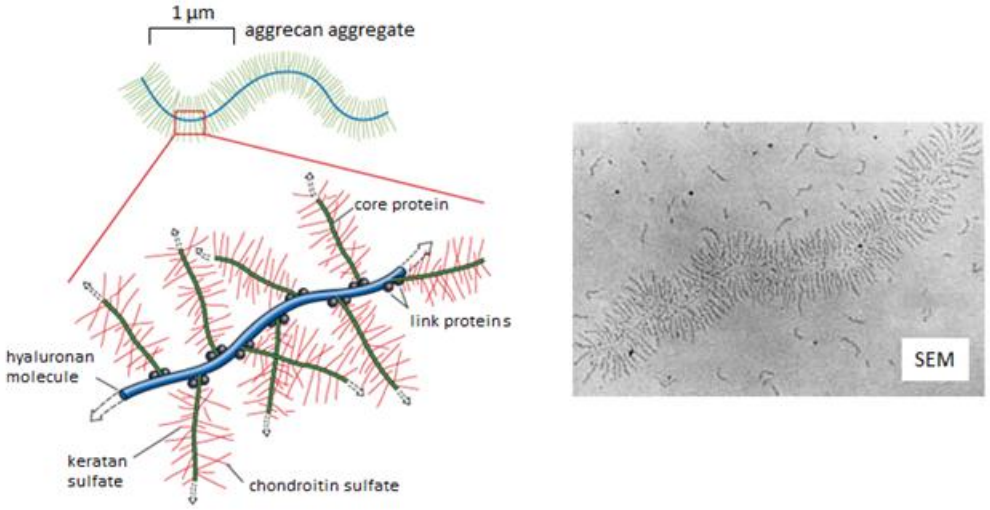

Figure 11. The aggrecan is the main proteoglycan of the articular cartilage characterized by a dendrigraft structure, as shown in scanning electron microscopy (SEM) image. Its hyaluronan backbone links peptide side chains which in turn bear polysaccharides (i.e. keratan or chondroitin sulfate).

Proteoglicans inspired the synthesis of polymer with similar architectures. These synthetic counterparts are known as cylindrical polymer brushes where the substrate is constituted by a polymer chain backbone. The architecture of molecular brushes can vary, being densely or loosely grafted, having flexible or stiff side chains and backbone, being homopolymers or copolymers (Figure 12). Each of these variables affect their physicochemical properties. The most important parameter which affects conformations and properties of polymer brushes is the steric repulsion between the densely grafted side chains. This peculiar characteristic enhances stiffness of the backbone, avoiding overlapping and entanglement with neighboring macromolecules, and promotes ordering.[24]

There are three main synthetic route for preparing densely grafted polymer brushes (Figure 13): *grafting through* (the polymerization of macromonomers), *grafting onto* (the formation of the graft copolymer originates from the coupling reaction between the functional backbone and the end-groups of the branches that are reactive) and *grafting from* (the growth of side chains from a macroinitiator backbone with a predetermined number of initiation sites).[24]

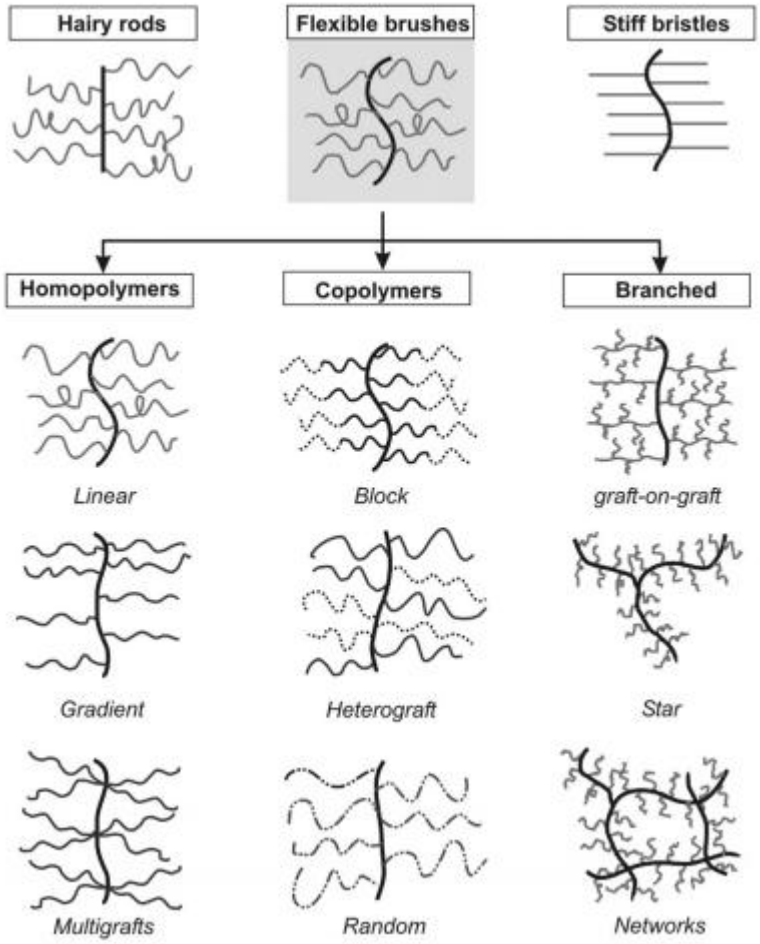

Figure 12. Various branching topologies and chemical compositions of cylindrical brushes.[24]

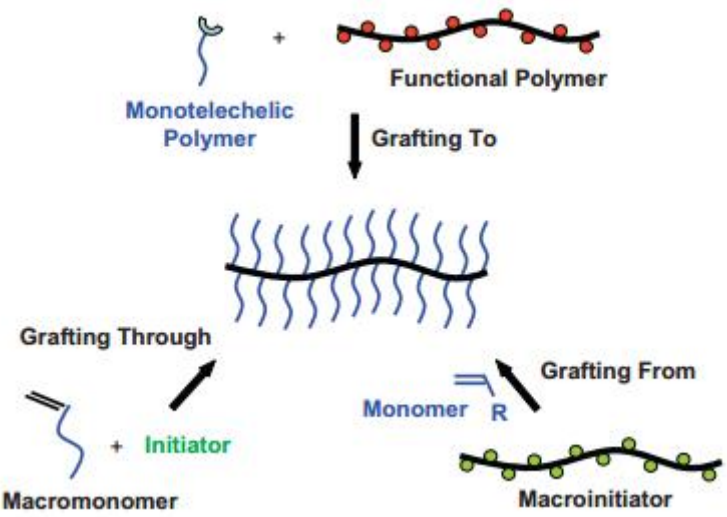

Figure 13. Three main strategies for preparing molecular brushes: grafting through, grafting onto, and grafting from.[24]

Polymer brushes first gained attention in the 1950s when it was discovered that flocculation could be prevented by grafting polymer molecules to colloidal particles.[25-30] Subsequently it was found that polymer brushes can be useful in other applications. In particular, they are used to stabilize colloids, reduce friction between surfaces and provide lubrication in artificial joints.[31]

In addition, polymer brushes are sensitive to variations of the surrounding environment (i.e. temperature, pH, solvent quality, ionic strength) and can undergo a dramatic physical or chemical change in response to external stimuli. This peculiar behaviour may be suitably applied in pharmaceutical field to develop new responsive DDSs.[23]

Conducting polymers

Traditionally, polymers are thought as insulators. In fact, they have always been used for their interesting chemical and mechanical properties and not for their electronic characteristics. The use of polymers as electronic materials is relatively recent. In 1977 a discovery by Alan G. MacDiarmid, Hideki Shirakawa, and Alan J. Heeger et al. changed the traditional concept. They found that suitably doped polyacetylene (PA) showed a conductivity value comparable to that of good metal conductors, such as copper and silver, whereas in its original form it is a semiconductor.[32] Soon after this discovery, a series of stable conducting polymers, including polypyrrole (PPy), polyaniline (PAn), polythiophene (PTh), poly(p-phenylene vinylene) (PPV), poly(p-phenylene) (PPP) and polyfluorene (PF) were reported, which greatly promoted the research on conducting polymers (Figure 14).[33]

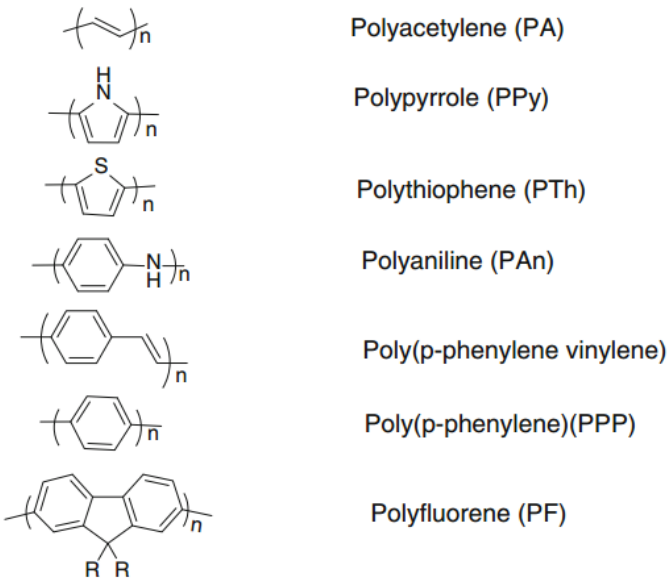

Figure 14. Main chain structures of several representative conjugated polymers.[33]

Conductive polymers or, more precisely, intrinsically conducting polymers (ICPs) are organic polymers that conduct electricity.[34] Actually, the conductivity of almost all conjugated polymers can reach the order of 10^{-3}–10^{3} S/cm after doping. Among ICPs, polyacetylene remains the one with the highest conductivity (10^{5} S/cm), but its high sensitivity to air and moisture make it unsuitable for practical uses. Although the other products have a lower conductivity, they are much more stable and useful for many practical applications.[33]

The conductivity of such polymers is the result of several processes. For example, in traditional polymers such as polyethylenes, the valence electrons are bound in sp^3 hybridized covalent bonds. Such " σ-bonding electrons" have low mobility and do not contribute to the electrical conductivity of the material. However, in conjugated materials the situation is completely different. Conducting polymers have backbones of contiguous sp^2 hybridized carbon centers. One valence electron on each center resides in a *pz* orbital, which is orthogonal to the other three σ-bonds. All the *pz* orbitals combine with each other to a molecule wide delocalized set of orbitals. This results in the formation of π-delocalized states along the polymer chain. The electrons in these delocalized orbitals may have high mobility when the material is "doped" by oxidation (or reduction). This doping process leads to the formation of charged species with high conductivity.

Differently, undoped conjugated polymers state are semiconductors with low electrical conductivity (Figure 15). Upon doping, electrical conductivity increases several orders of magnitude.[33]

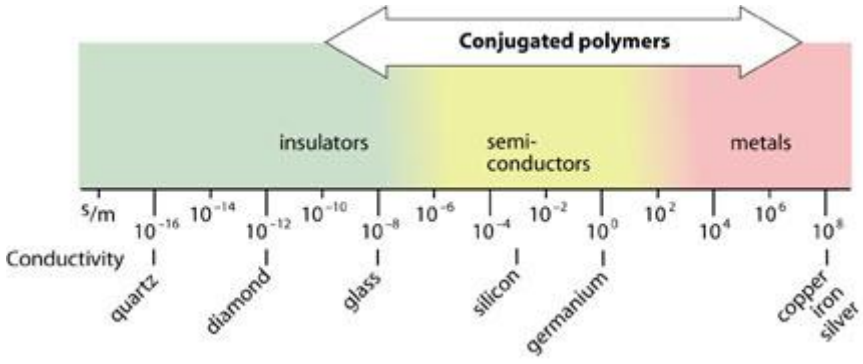

Figure 15. Conductivity of some materials as compared to conjugated polymers.[35]

Conjugated polymers possess a doped conducting state and a neutral semiconducting state, which lead to different applications. The doped conducting state could be found applications in the fields of electrode materials for batteries, electrochromics and super-capacitors, anti-static and anti-corrosion materials, electrolyte capacitors, transparent electrodes, chemical and biosensors, etc. The neutral (intrinsic) semiconducting state makes these materials applicable in polymer light emitting diodes (PLEDs), polymer solar cells (PSCs), polymer field effect transistors (FETs), etc.[33]

The main advantages of conducting polymers are that they possess not only the electronic and optical properties of metals and inorganic semiconductors, but also the flexible mechanics and processability of polymers. In addition, there is special electrochemical redox activity with conducting polymers.[33] However, in comparison with inorganic semiconductors for optoelectronic applications, conjugated polymers possess some drawbacks such as poorer stability, lower charge carrier mobility, difficulty in forming ordered structure, etc.[33] In order to overcome these drawbacks a large variety of π-conjugated polymers have been synthesized, but a little consideration has been devoted to through-space conjugated polymers, which show stacked arrays of π-electron systems along the polymer backbone.[36] The most investigated π-stacked polymer is poly(N-vinylcarbazole) (PVK, Figure 16), which has been demonstrated to transport charge via intrachain stacked orbitals that result from face-to-face conformation of the carbazole moieties, and shows photoconductive, photorefractive, hole-transporting, energy-donating, and carrier-switching properties.[37]

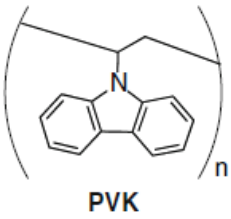

Figure 16. Structure of PVK.

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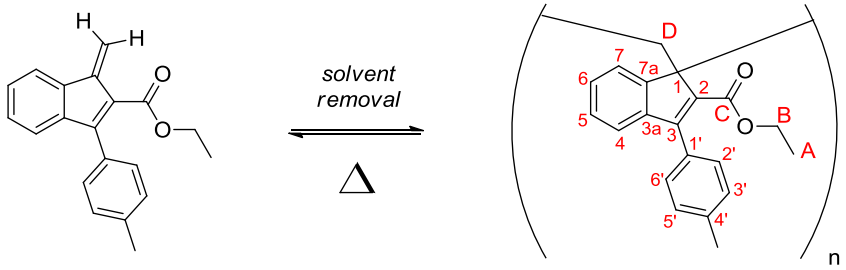
Polybenzofulvenes

Definition and Properties

Polybenzofulvenes represent a new class of polymeric materials showing intriguing features potentially useful for pharmaceutical and optoelectronic applications.

Studies carried out on the prototypical poly-**BF1** (Scheme 1) have shown interesting properties such as the rapid and almost quantitative formation by spontaneous thermoreverible polymerization, high degree of π-stacking, tendency to generate nanostructured macromolecular aggregates, high solubility in the most common organic solvents, significant level of biocompatibility, predisposition towards molecular manipulation.[1,2]

Scheme 1

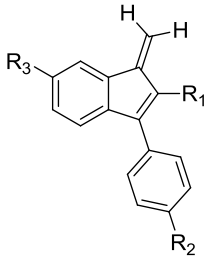


BF1 poly-**BF1**

In order to evaluate the effects of the introduction of specific substituents having the appropriate steric and electronic characteristics on the structure-property relationships of polybenzofulvene derivatives, several molecular modifications have been made in precise positions of the 3-phenylindene nucleus of poly-**BF1**. These efforts have produced the series of benzofulvene derivatives **BF3** (Table 2).[1]

In particular, it was observed that the spontaneous polymerization results to be hindered by the presence of sterically bulky groups ($CON(CH_3)_2$ or $C(CH_3)_3$) located on the carbon C_2 of the indenic ring. Furthermore, the type of polymerization results to be affected by both the dimension and the position of substituents. In fact, the presence of relatively bulky groups on the carbon C_2, such as COOEt, favor the vinyl (1.2) polymerization, while smaller functional groups (H, F, Cl, Br, CN) lead to the formation of heterogeneous polymer chains, along which (1,2) and (1,4) sequences can coexist (Figure 17). Finally, the presence of less hindered residues (i.e. CH_3) in position 6 direct the process towards the vinyl (1.2) polymerization.[1]

Table 2. Benzofulvene derivatives **BF3**.[1]



BF3 derivatives

Monomer	R_1	R_2	R_3
BF3a	H	H	H
BF3c	F	H	H
BF3d	Cl	H	H
BF3e	Br	H	H
BF3f	CH_3	H	H
BF3g	CN	H	H
BF3h	CN	CH_3	H
BF3i	CN	H	CH_3
BF3j	C≡C-2-Pyr	H	H
BF3k	$COOC_2H_5$	H	H
BF3ma	$COOC(CH_3)_3$	CH_3	H
BF3n	$CON(CH_3)_2$	CH_3	H
BF3o	$C(CH_3)_3$	H	H

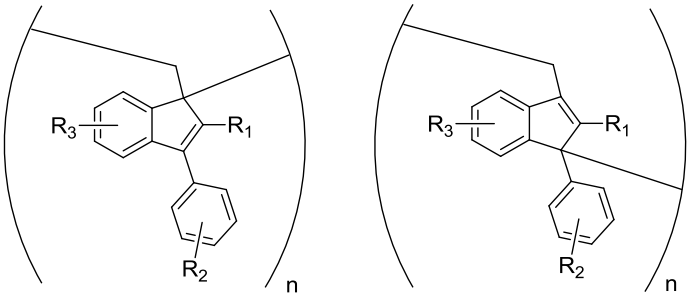


Figure 17. Structure of polybenzofulvenes derived from the two possible enchainments afforded by either a vinyl (1,2) polymerization (**left**) or a diene (1,4) polymerization (**right**) of the corresponding monomers.

Polymerization mechanism

The added value of this new class of polymers is constituted by the spontaneous polymerization by simple solvent removal starting from the solutions of the respective monomers. Moreover, the absence of catalysts and/or initiators used to trigger the polymerization, makes polibenzofulvenes not contaminated by any traces of extraneous agents. This constitutes a considerable advantage for their potential application in pharmaceutical field.

The monomer **BF1** has been shown to polymerize also in the presence of radical inhibitors such as 2,6-di-tert-butyl-p-cresol, whereas it forms Michael-type (1,2 and 1,4) byproducts in the presence of a nucleophile (i.e. N-methylpiperazine, NMP). It was hypothesized an ionic polymerization mechanism based on the polar character of the exocyclic double bond of **BF1**. Thus, we assumed that the interaction of the strongly polarized exocyclic double bond of two **BF1** molecules could produce the formation of an activated dimer featuring a zwitterionic tetramethylene moiety (Figure 18) where the negative charge is stabilized as an aromatic indenyl anion while the positive charge is localized on the terminal methylene (CH_2^+). The latter could initiate the cationic polymerization process leading to the corresponding polymer.[2]

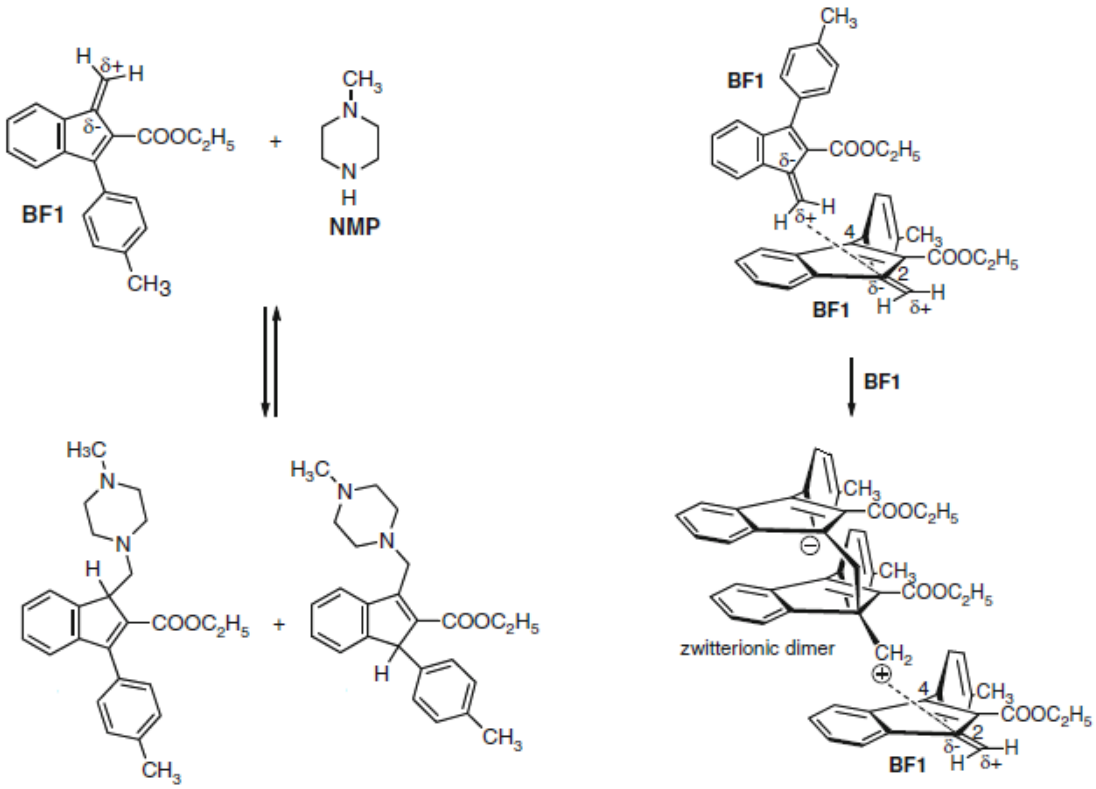


Figure 18. Left. Reaction of benzofulvene derivative **BF1** with N-methylpiperazine (NMP). **Right.** Earlier mechanistic hypothesis for the spontaneous polymerization of **BF1**.[2]

A second mechanistic hypothesis takes into account the strong tendency of benzofulvene derivatives to give π-stacking interactions. This property allows these hydrophobic derivatives to self-assemble in columnar-shaped aggregates up to the critical distance of polymerization. Furthermore, the obtaining of homopolymer poly-**BF1** by concentration of the monomer solution in the presence of other vinylic molecules (i.e. Styrene) supports this mechanistic hypothesis, suggesting that the polymerization process can be based on a very selective molecular recognition phenomenon (affinity polymerization).

Furthermore, poly-**BF1** has been obtained also by means of anionic polymerization (**AP**) in the presence of phenyl lithium as initiator. This material (poly-**BF1-AP**) has shown a lower molecular weight (Mw = 7.4 kg/mol) than the one prepared by spontaneous polymerization (poly-**BF1-SP**, Mw = 2670.0 kg/mol). The two macromolecules are further different in relation to the tendency to aggregation. In fact, poly-**BF1-AP** has appeared to be liable to give nanospheres and microspheres showing favorable shapes and dimensions, while poly-**BF1-SP** is prone to generate larger fractal-like aggregates (Figure 19).[3]

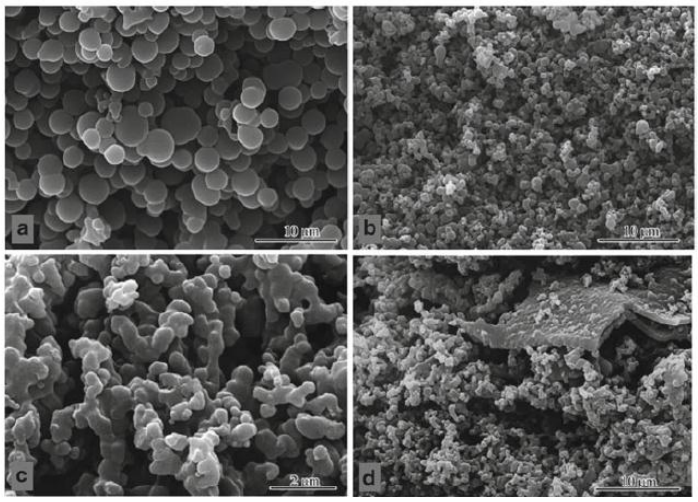


Figure 19. Molecular aggregate structure found by SEM analysis of poly-**BF1** precipitated from a dichloromethane solution with ethanol. (**a**) Microspheres obtained with poly-**BF1**-**AP**; (**b**) nanoparticles obtained with poly-**BF1**-**AP**; and (**c**) and (**d**) more complex aggregates obtained with poly-**BF1**-**SP** shown at different magnification ratios.[3]

PEGylated polybenzofulvene brushes

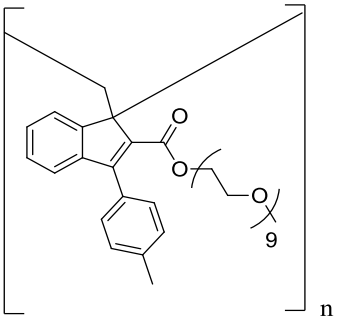
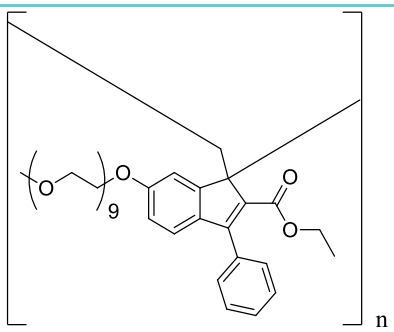
Nevertheless, the low solubility in water of the first polybenzofulvene derivatives was one of the main limitations for their application in the pharmaceutical field. In order to obviate to this problem a

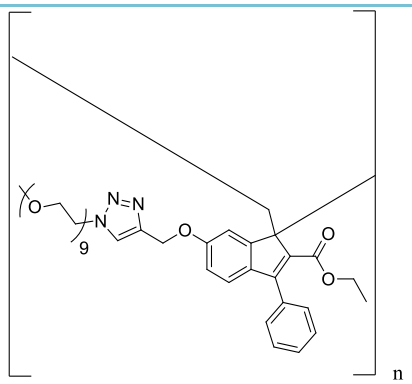
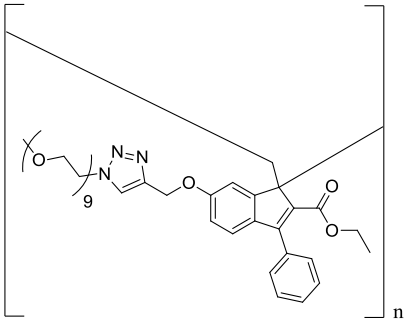
number of structural changes such as the inclusion of monomethyl-oligo (ethylene glycol) chains (MOEG) onto the polibenzofulvene backbone were made.[4-6]

Poly(ethylene glycol) PEG confers a better solubility in the physiological environment and prevents the interaction with plasma proteins and cells by virtue of its valuable features, such as water-solubility, biocompatibility, lack of toxicity and shielding properties preventing opsonization (i.e. stealth PEGylated liposomes). It can be used either as a drug carrier or covalently linked to bioactive molecules for their in vivo delivery.[6,7]

This approach afforded the family of the polybenzofulvene molecular brushes (PBFMBs) (Table 3).

Table 3. Polybenzofulvene brushes synthesized in the last years.

Polymer	Structure and Synthesis	Behavior in water
Poly-2-MOEG-9-**BF1**	 ***GRAFTING THROUGH*** Spontaneous polymerization of the corresponding monomer	Formation of a compact physical hydrogel, stable when exposed to ultrasounds
Poly-6-MOEG-9-**BF3k**	 ***GRAFTING THROUGH*** Spontaneous polymerization of the corresponding monomer	Formation of a physical hydrogel, which can be weakened by means of ultrasounds

Poly-6-MOEG-9-TM-**BF3k-GT**	 ***GRAFTING THROUGH*** Spontaneous polymerization of the corresponding monomer	Gradual dissolution in water by means of ultrasounds
Poly-6-MOEG-9-TM-**BF3k-GO**	 ***GRAFTING ONTO*** Post-polymerization functionalization	Gradual dissolution in water by means of ultrasounds

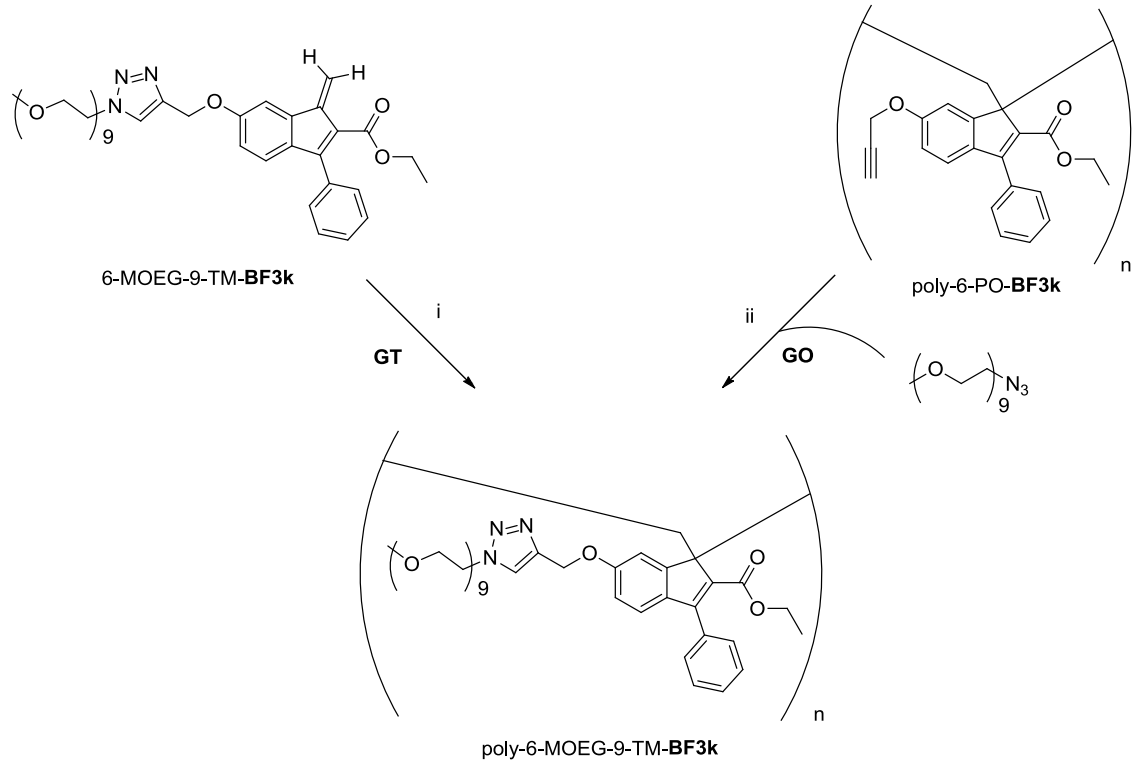
All PEGylated polybenzofulvene brushes (PPBFBs) reported in Table 3 are soluble in the most common organic solvents, while a different behavior of this polymers was observed in water.

Poly-2-MOEG-9-**BF1** and poly-6-MOEG-9-**BF3k** were designed starting from poly-**BF1** and its derivative poly-6-MO-**BF3k**, respectively, using suitable coupling reactions in pre-polymerization step (grafting through approach). Nevertheless, even if the grafting of relatively long MOEG-9 side chains in poly-**BF1** and poly-6-MO-**BF3k** macromolecules allowed to these polymers to interact with water, the length of the side chains was found to be inadequate to warrant a complete dissolution in the aqueous environment. [5,6]

In order to enhance the hydrophilic character of polybenzofulvene derivatives was investigated the insertion of a triazolomethyl (TM) spacer with the aim of separating the hydrophilic domain (MOEG-9 side chains) from the hydrophobic one (polybenzofulvene backbone). Thus, a new polymer brush was synthesized by means of an orthogonal strategy of conjugation to covalently link MOEG-9 side chains to the benzofulvene nucleus, in which the copper(I)-catalyzed alkyne-azide 1,3-dipolar

cycloaddition (CuAAC) click reaction and the spontaneous polymerization process were combined both in a grafting through (**GT**) and in a grafting onto (**GO**) approach, in order to obtain the derivative poly-6-MOEG-9-TM-**BF3k** (Scheme 2). We assume that the insertion of a triazolomethyl spacers increases the efficiency of the interaction between MOEG-9 pendant residues (hydrophilic domain) and water by separating the hydrophilic domain from the hydrophobic one (polybenzofulvene backbone). The grafting onto approach represents, from a synthetic point of view, the easiest way to produce PPBFBs with a molecularly dispersed degree and a reduced tendency to aggregate.[4]

Schema 2



Reagents: (i) rimozione del solvente; (ii) CuBr, DIPEA, THF.

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Aims of the thesis

During my PhD studies I worked on the synthesis and characterization of new polymeric materials for pharmaceutical and optoelectronic applications. Particular attention was given to the benzofulvene monomers that, through a mechanism of spontaneous polymerization by simple solvent removal provide polymers with interesting properties, such as: thermoreverible polymerization/depolymerization behavior, high degree of π-staking, tendency to generate nanostructured macromolecular aggregates, significant level of biocompatibility, high solubility in the most common organic solvents, predisposition towards molecular manipulation. Moreover, the absence of catalysts and/or initiators used to trigger the polymerization makes polibenzofulvenes not contaminated by any traces of extraneous agents.

Nevertheless, the low solubility in water of the first polybenzofulvene derivatives was one of the main limitations for their application in the pharmaceutical field. In order to obviate to this problem a number of structural changes such as the inclusion of monomethyl-oligo (ethylene glycol) chains (MOEG) onto the polibenzofulvene backbone were made. This approach afforded the family of the polybenzofulvene molecular brushes (PBFMBs). The ability shown by PBFMBs to load and then deliver bioactive molecules by means of unspecific interactions together with the above mentioned interesting properties make polybenzofulvenes excellent candidates for the development of new drug delivery systems.

With the aim of obtaining new advanced functional materials capable of recognizing/loading and then delivering small drug molecules by means of non-covalent interactions, the polybenzofulvene backbone of molecular brush poly-6-MOEG-9-TM-**BF3k** has been functionalized with a "synthetic dynamic receptor" composed of two 1-adamantylurea moieties linked together by means of a dipropyleneamino bridge as in Meijer's bis(adamantylurea) pincer (BAUP). To evaluate the effects of the introduction of BAUP into the polybenzofulvene environment homopolymer poly-6-BAUP-TM-**BF3k** and copolymer poly-6-MOEG-9-TM-**BF3k**-co-6-BAUP-TM-**BF3k** (9:1) were prepared, both equipped with synthetic receptors but with different densities (see **Chapter 2**). In particular, the copolymer was used to prepare colloidal drug delivery systems for delivering the model anticancer doxorubicin (**DOXO**).

Subsequently, based on the pivotal role played by the hyaluronic acid (HA) in many cellular functions and the largely recognized overexpression of its CD44 receptor in tumor tissues, the family of the polybenzofulvene molecular brushes was enriched by the tri-component polymer brush (**TCPB**) composed of a polybenzofulvene backbone bearing nona(ethylene glycol) (NEG) arms terminated with low molecular hyaluronic acid macromolecules. This work was aimed at developing new

biomimetic multifunctional materials capable of forming biocompatible aggregates for biomedical applications (i.e. drug delivery systems). Moreover, the presence of side chain arms terminated with low molecular weigth HA macromolecules can confer to the resulting nanoaggregates targetability towards tumor cells expressing the CD44 receptor.

In particular, as shown in **Chapter 2**, **TCPB** was synthesized by means of a convergent approach involving three different steps: the synthesis of the polybenzofulvene backbone bearing nona(ethylene glycol) side chains terminated with a clickable azido groups, the functionalization of low molecular weight hyaluronic acid macromolecules with ferulic acid bearing clickable propargyl groups, and the coupling between the two macromolecular synthons by copper(I)-catalyzed azide-alkyne 1,3-dipolar cycloaddition.

Furthermore, in order to obtain new polymeric materials endowed with improved optoelectronic performances, we envisioned the insertion of different chromophores [i.e. 9,9-dimethylfluorene (DMFL), triphenylamine (TPA), 9-methylcarbazole (MCBZ), 2,2'-bithiophene (BT) and 5-hexyl-2,2'-bithiophene (HBT) residues] in two different key positions (6 and 4') of the 3-phenylindene scaffold of the polybenzofulvene monomeric units (see **Chapter 3**). We have shown that polybenzofulvene derivatives represent a new family of π-stacked polymers showing interesting hole-mobility features. The absorption and emission features of this class of polymers do not vary significantly from the solid state to diluted solutions, both characterized by quite large Stokes shifts. All these characteristics make polybenzofulvene derivatives excellent candidates for the development of more complex nanostructured films with enhanced optical and optoelectronic properties (such as organic light emitting diodes, OLEDs).

Chapter 2.

Polybenzofulvenes for Drug Delivery Systems

- **Polybenzofulvene derivatives bearing dynamic binding sites as potential anticancer drug delivery systems**

Andrea Cappelli, Giorgio Grisci, Marco Paolino, Vincenzo Razzano, Germano Giuliani, Alessandro Donati, Claudia Bonechi, Raniero Mendichi, Antonella Caterina Boccia, Mariano Licciardi, Cinzia Scialabba, Gaetano Giammona and Salvatore Vomero.

(Front Cover of Volume 3, Number 3, 21 January 2015, Pages 333–516).

Journal of Materials Chemistry B, **2015**, 3, 361-374.

DOI: 10.1039/c4tb01268b

- **Hyaluronan-coated polybenzofulvene brushes as biomimetic materials**

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Polym. Chem., **2016**, 7, 6529-6544.

DOI: 10.1039/c6py01644h

Chapter 3.

Polybenzofulvenes for Optoelectronic Applications

- **Highly emissive supramolecular assemblies based on π-stacked polybenzofulvene hosts and a benzothiadiazole guest**

Andrea Cappelli, Francesca Villafiorita-Monteleone, Giorgio Grisci, Marco Paolino, Vincenzo Razzano, Giuseppe Fabio, Germano Giuliani, Alessandro Donati, Raniero Mendichi, Antonella Caterina Boccia, Mariacecilia Pasini and Chiara Botta.

Journal of Materials Chemistry C, **2014**, 2, 7897-7905.

DOI: 10.1039/c4tc01200c

- **π-Stacked polybenzofulvene derivatives as hosts for yellow and red emitting OLEDs**

Wojciech Mróz, Francesca Villafiorita-Monteleone, Mariacecilia Pasini, Giorgio Grisci, Marco Paolino, Vincenzo Razzano, Andrea Cappelli, Chiara Botta.

Materials Letters, **2015**, 142, 197-200.

http://dx.doi.org/10.1016/j.matlet.2014.12.002

- **Bithiophene-based polybenzofulvene derivatives with high stacking and hole mobility**

Andrea Cappelli, Vincenzo Razzano, Marco Paolino, Giorgio Grisci, Germano Giuliani, Alessandro Donati, Raniero Mendichi, Filippo Samperi, Salvatore Battiato, Antonella Caterina Boccia, Andrea Mura, Giovanni Bongiovanni, Wojciech Mróz, Chiara Botta.

(Front Cover of Volume 42, Number 6, 14 November 2015, Pages 7355–7486).

Polymer Chemistry, **2015**, 6, 7377-7388.

DOI: 10.1039/c5py00904a

- **Side chain engineering in π-stacked polybenzofulvene derivatives bearing electron-rich chromophores for OLED applications**

Andrea Cappelli, Vincenzo Razzano, Giuseppe Fabio, Marco Paolino, Giorgio Grisci, Germano Giuliani, Alessandro Donati, Raniero Mendichi, Wojciech Mr´oz, Francesca Villafiorita-Monteleone and Chiara Botta.

RSC Adv., **2015**, 5, 101377–101385.

DOI: 10.1039/c5ra21164f

Curriculum vitae

Vincenzo Razzano was born on 8th September 1988 in Cariati (CS, Italy). After finishing secondary school at the "Liceo Scientifico Tecnologico I.T.I. Tito Sarrocchi" (Siena, SI, Italy), he started his studies in Medicinal Chemistry at the University of Siena in 2007. In order to complete his studies, he spent one year as an undergraduate researcher in the group of Prof. Fabio Ponticelli and finished his graduation thesis on "Indanylidenpyrrolinic molecular photoswitches derivatized with amino groups". The synthesis and characterization of molecular photoswitches activated by light irradiation and the photochemical studies on synthesized molecules provided him opportunities to learn the principles of Organic Chemistry and Photochemistry as well as analytical techniques such as Flash Column Chromatography, Thin Layer Chromatography (TLC), Nuclear Magnetic Resonance Spectroscopy (NMR), Liquid Chromatography-Mass Spectrometry (LC-MS), Infrared Spectroscopy (IR) and the basic principles of X-ray diffraction (XRD).

Following his graduation, he worked for six months at Aptuit (Verona), Italy. Through this period, he had experiences in Medicinal Chemistry improving his capabilities in the synthesis and characterization of organic compounds, as well as concepts of safety in the workplace and Risk-Assessment. He also learnt other analytical techniques such as the Ultra Performance Liquid Chromatography (UPLC).

Then he received fundings for his doctorate studies in Chemical and Pharmaceutical Science from the University of Siena. His Ph.D. was done under the joint supervision of Prof. Andrea Cappelli. Throughout his research, he acquired knowledge in Polymer Chemistry. His work was focused on the synthesis and characterization of new polymeric materials for pharmaceutical and optoelectronic applications. He also acquired knowledge in Fluorescence Spectroscopy and other analytical techniques such as the High-Performance Liquid Chromatography (HPLC). Furthermore, he enhanced his capabilities in the synthesis and characterization of organic compounds as well as purification techniques by means of crystallization and precipitation.

Pubblications:

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Polym. Chem., **2016**, 7, 6529-6544.

Acknowledgements

In the final pages, I would like to acknowledge some people who helped me during my thesis work.
First of all, I would like to thank my academic supervisor Prof. A. Cappelli who gave me the opportunity to work in his group. You have allowed me both to grow up as a man and to enlarge my scientific knowledge by creating a friendly and homely environment.
Furthermore, I would like to say "thank you" to Dr. M. Paolino for his advice and patience shown over these years. You have also contributed to improve myself and my skills.
I cannot forget my research group (Prof. M. Anzini, Dr. G. Giuliani) and especially all the graduate and undergraduate students. Not only have you been my colleagues but, more importantly, you have also become my new friends.
Many thanks also to RottapPharm S.p.A. for financing my PhD thesis.
Thanks to Prof. A. Donati for collaborating in NMR studies.
Thanks to Dr. C. Botta for photophysical studies.
Thanks also to Prof. G. Giorgi and Dr. F. Berrettini for X-Ray crystallography analysis.
Last but not least, thanks to my family for their support and strength and for teaching me to keep going and never give up.
Finally, I want to thank my girlfriend Federica for always being with me and for making me feel a special person.